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DISSIMILATION OF C^{14}-LABELED GLUCOSE BY *SERRATIA MARCESCENS*

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Glucose containing radioactive carbon has been utilized in the study of the metabolic distribution of the various carbon atoms during dissimilation by the following organisms: *Leuconostoc mesenteroides*, (Gunsalus and Gibbs, J. Biol. Chem. **194,** 871, 1952); *Pseudomonas saccharophila*, (Entner and Doudoroff, J. Biol. Chem. **196,** 853, 1952); *Pseudomonas fluorescens* (Lewis *et al.*, J. Biol. Chem., **216,** 273, 1955). The distribution of the radioactivity of variously labeled glucose-C^{14} after oxidation by *Serratia marcescens* is reported herein.

S. marcescens, grown on a dry milk-glucose medium (Wasserman *et al.*, Can. J. Microbiol. **2,** 447, 1956), was allowed to oxidize glucose-1-C^{14}, glucose-6-C^{14} (obtained from the National Bureau of Standards), and glucose-2-C^{14} (obtained from Tracerlab[2]). The experiments, conducted in the Warburg respirometer, were concluded when the rate of oxygen consumption in the presence of substrate was the same as that of the endogenous cells. Carbon dioxide, collected in 5 N NaOH in the center well, was precipitated as $BaCO_3$. The cells were separated from the supernatant by centrifugation and both fractions converted to CO_2 by the persulfate method (Calvin *et al.*, *Isotopic Carbon*, J. Wiley and Sons, 1949). The precipitated $BaCO_3$ was collected on filter paper disks using the Tracerlab precipitation apparatus and counted with an accuracy of 5 per cent. The counts were corrected to infinite thinness. Pyruvic acid was determined by the Friedemann and Haugen method (J. Biol. Chem., **147,** 415, 1943), and 2-ketogluconic acid by the method of Lanning and Cohen (J. Biol. Chem., **189,** 109, 1951).

Table 1 shows the distribution of radioactivity following dissimilation of labeled glucose by *S. marcescens* in the absence and presence of sodium arsenite. At least 60 per cent of 1-C of glucose is converted to CO_2 by decarboxylation, and this reaction is unaffected by the inhibitor. Twenty-four per cent of the activity of glucose-1-C^{14} is localized within the cell by some pathway of assimilation which is sensitive to the arsenite inhibitor.

TABLE 1

Distribution of radioactivity following dissimilation of labeled glucose by Serratia marcescens

Labeled Glucose	% Original Activity			μMoles Formed	
	CO_2	Cells	Supernatant	Pyruvic	2-KG *
1-C	60.0	24.0	13.4	0	0
1-C+ Arsenite	60.0	7.2	36.4	1.8	0.55
2-C	41.0	40.0	19.6	0	0
2-C+ Arsenite	29.2	14.4	53.0	1.6	0.60
6-C	27.5	54.5	16.8	0	0
6-C+ Arsenite	3.2	13.2	84.0	1.7	0.65

Each flask contained 0.5 ml 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.2; 100 μg phosphorus; *S. marcescens* (4 mg dry wt); 2.5 μmoles substrate; 0.1 ml 0.1 M sodium arsenite, where indicated; water to 2 ml.

Glucose-1-C^{14} = 11,500 cpm; glucose-2-C^{14} = 10,500 cpm; glucose-6-C^{14} = 10,500 cpm.

* 2-KG = 2-ketogluconic acid.

The distribution of carbon-6 probably reflects the fate of the last three carbon atoms of glucose. In the absence of inhibitor, 54.5 per cent of the activity of C-6 was in the cellular fraction and 27.5 per cent appeared as CO_2. It is presumed

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[2] Mention of a commercial firm does not constitute endorsement by the U. S. Department of Agriculture over similar firms not mentioned.

that this three carbon fragment is metabolized via pyruvate, since arsenite inhibition prevents the labeled carbon-6 from being eliminated as CO_2 and reduces considerably the quantity incorporated within the cell.

The data show that labeled carbon-2 is metabolized via both arsenite sensitive and insensitive pathways. In the absence of arsenite, 41 per cent of the activity occurs as CO_2 and 40 per cent is found within the cell. However, in the presence of the inhibitor, 29 per cent of the activity is recovered as CO_2 and only 14 per cent is found intracellularly.

In the absence of arsenite, approximately 17 per cent of the activity of any of the labeled forms of glucose was recovered from the supernatant. However, unpublished experiments indicated that all glucose had disappeared from the medium prior to the completion of the experiment. The nature of the compound(s) containing the residual activity could not be identified by the chemical and paper chromatographic tests for the conventional intermediates utilized.

Chemical determination for 2-ketogluconic acid and pyruvic acid indicated 0.6 μmole of the former and 1.7 μmoles of the latter were formed from 2.5 μmoles glucose in the presence of arsenite, but not in its absence. The concentrations of 2-ketogluconic acid and pyruvic acid were not great enough to account for the activity in the medium. However, it has been reported previously (Wasserman *et al.*, Can. J. Microbiol. **2,** 447, 1956) that an unidentified acidic component accumulated in the medium in the presence of arsenite, and it is possible that the residual activity may be located in this compound.

Since this project has been terminated, further work in defining the metabolic breakdown of glucose by *S. marcescens* is not contemplated in this laboratory.